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Biological Contribution

Translational Aspects of Nuclear Factor-Kappa B and Its Modulation by Thalidomide on Early and Late Radiation Sequelae in Urinary Bladder Dysfunction

Jakob Kowaliuk, MSc,* Sina Sarsarshahi, MSc,*,† Johanna Hlawatsch, BSc,* Alexandra Kastsova,* Maria Kowaliuk, MSc,* Alexander Krischak, Mag.med.vet.,*,§ Peter Kuess, PhD,‡ Lisa Duong,* and Wolfgang Dörr, Univ.-Prof. Dr*

**ATRAB-Applied and Translational Radiobiology, Medical University of Vienna, Vienna, Austria; †Department of Molecular Medicine, Iran University of Medical Science, Tehran, Iran; ‡Division of Medical Physics, Department of Radiation Oncology, Medical University of Vienna, Vienna, Austria; and §Platform Radiooncology and Nuclear Medicine, Department for Companion Animals and Horses, University of Veterinary Medicine of Vienna, Vienna, Austria*

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Purpose: This preclinical study aimed to investigate the role of nuclear factor (NF)-κB in early and late radiogenic sequelae of urinary bladder dysfunction in mice. Thalidomide was applied either during the early or late response phase to determine potential effects of NF-κB inhibition on functional bladder impairment.

Methods and Materials: After pelvic irradiation on day 0, female C3H/Neu mice were observed over a period of 360 days and radiation response was evaluated for alterations in bladder functionality and NF-κB activation. Functionality was determined in graded dose experiments (14-24 Gy) and assessed by micturition frequency analysis and transurethral cystotonometry to reveal alterations in voiding and volume. The induction of the NF-κB proteins p50 and p65 was evaluated by immunohistochemistry in response to a single dose of 23 Gy (ED_{90}). Thalidomide (100 mg/kg/d) was applied intraperitoneally in 3 treatment groups: daily from day 1 to 15, daily from day 16 to 30, and in 2-day-intervals from day 150 to 180.

Results: Immunohistochemical analysis showed a biphasic activation of p50 and p65 during the early radiation cystitis phase (day 1-30). After a transient decrease, p50, but not p65, was reactivated permanently leading to increased levels, which suggests an occurrence of chronic inflammation correlated with functional impairment. Both early thalidomide treatments reduced NF-κB activation and shifted the ED_{50} value for early radiation cystitis and late radiation sequelae to higher doses.

Conclusions: These data clearly demonstrate the involvement of NF-κB signaling in the pathogenesis of radiation-induced urinary bladder dysfunction. Additionally, this study emphasizes that biological targeting of early radiogenic processes has enormous effect on chronic symptoms. The late administration of thalidomide showed no significant effect on functionality.

Corresponding author: Jakob Kowaliuk, MSc; E-mail: jakob.kowaliuk@meduniwien.ac.at

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Introduction

During the course of pelvic radiation therapy, the urinary bladder is exposed to particular doses that bear a certain, but accepted, risk of adverse effects in favor of tumor control.[1] Radiogenic complications of the urinary bladder are divided into early and late events. Early symptoms mainly reported days to weeks after the onset of radiation therapy are reversible and undergo conservative treatments.[2] However, a progressive and irreversible late phase is preceded by a symptom-free period that persists for years or even decades.[3,4] The urologic symptoms comprise a tremendous reduction of the bladder volume, leading to an increased micturition frequency and nocturia, and include dysuria, hematuria, and incontinence. In consideration of continuously increasing curing rates, it is important to manage chronic symptoms to further improve the patient's quality of life.

So far, no adequate treatment strategies exist to prevent, reduce, or cure late radiation sequelae (LRS). The current management of early radiation cystitis (ERC) indicates no ameliorating effect on late complications, although insights into the radiopathogenesis reveal a fundamental relationship between early and late radiation responses, known as consequential (late) effects.[2,3,5] A biology-based intervention that targets early radiation-induced mechanisms offers a sophisticated approach to mitigate early radiation sequelae and presumably LRS.

Radiation-induced reactions in the urothelium provoke excessive inflammation that, in combination with an impairment of the barrier function, triggers early symptoms.[6,7] Preclinical studies demonstrated a biphasic upregulation of COX-2,[7] postulated as (1) a direct consequence of ionizing irradiation[8] and (2) a result of barrier impairment.[9] The mediated synthesis of prostaglandins increases the tonus of the detrusor muscle, which reduces the bladder volume, leading to increased micturition frequency and accessory symptoms.[10] An incomplete compensation of radiation-induced urothelial cell depletion presumably fosters persistent leakage of noxious urine that drives the production of reactive oxygen species, inducing additional trauma and consequential (late) effects.

Nuclear factor κB (NF-κB) is a pivotal transcription factor that regulates immune cell activation and is associated with inflammatory processes.[11-13] Hence, NF-κB is also suspected to contribute to the development and progression of inflammation-promoted tumors, such as prostate cancer.[14,15] Ionizing radiation activates the NF-κB signaling cascade directly or via the induction of double-strand breaks and oxidative stress.[16,17] Activated NF-κB proteins form homo- and heterodimers to translocate to the nucleus and enable the transcription of versatile genes.[11,12,18-20] The 2 NF-κB proteins p50 and p65 preferentially form heterodimers that induce inflammatory gene activation.[21-23] Thalidomide interferes with the activation of NF-κB, rationalizing its utilization for various clinical applications ranging from leprosy to diverse malignancies.[24,25]

Based on the immunomodulatory effect of thalidomide, this study aims to investigate the role of NF-κB and the functional effect of thalidomide on radiogenic bladder dysfunction. Therefore, the activation of p50 and p65 was evaluated in single-dose experiments for 360 days and correlated with functional assessments in female C3H/Neu wild-type mice.

Methods and Materials

Animals and housing

All animal experiments were performed in accordance with the current laboratory animal welfare legislation with approval of the respective authorities (Federal Ministry of Education, Science and Research, file number BMWF-66.009/0148-WF/II/3b/2014).

Groups of 5 C3H/Neu mice per cage (Tecniplast IVC cages, Buguggiate, Italy) were kept on aspen wood bedding (ABEDD, Lab & Vet Service GmbH, Köflach, Austria) at 12/12-hour light/dark rhythm under specific pathogen-free conditions. The temperature was set to 22°C to 24°C at 45% to 65% humidity. Animals received standard mouse diet (Sniff Spezialdiäten GmbH, Soest, Germany) and sterile filtered water ad libitum. For functional studies (240 mice), each treatment group consisted of 60 animals split into 5 irradiation dose groups (12 animals per group). Eight hundred eighty-five animals were designated for immunohistochemistry (IHC) and divided into the following treatment groups: nonirradiated control group (216), irradiation control (216), thalidomide day 1 to 15 (216), thalidomide day 16 to 30 (141), and thalidomide day 150 to 180 (96). In total, 1125 animals were examined in this study.

Irradiation

Small animal pelvic irradiation was described in previous papers.[26] Briefly, groups of 5 to 6 anaesthetized mice (60 mg/kg pentobarbitone sodium, intraperitoneally) at the age of 10 to 12 weeks underwent single-dose irradiations with a YXLON MG325 x-ray device (YXLON International GmbH, Hamburg, Germany) operating at 200 kV and 20 mA. Animals were immobilized in a supine position, and the urinary bladders were positioned to a corresponding 0.9 cm^2 irradiation field. The bowel was gently moved out of the irradiation field, and the rest of the animal was shielded by a lead equivalent collimation with the following composition: 3 mm Be, 4 mm Al, and 0.6 mm Cu. The dose rate was approximately 1 Gy/min at the focus-to-surface distance of 45.5 cm. Dose homogeneity between the individual bladder positions deviated by ±3%.

Experimental design

Animals were irradiated on day 0. Thalidomide was systemically applied (100 mg/kg/d)[22] in 3 treatment groups daily from day 1 to 15, from day 16 to 30, or in 2-day intervals from day 150 to 180. For functional studies, groups of 12 mice received 14, 17, 19, 21, or 24 Gy single irradiation and 23 Gy (ED_{90}, dose at which 90% show functional response based on the volumetric endpoint) for immunohistochemical examinations. Transurethral cystotonometry (TC) was conducted every 3 days during the early phase (day 3-30) and every 30 days during the late (day 60-360) phase. Baseline values were measured within 2 weeks before irradiation. Micturition frequency analysis was conducted in the late phase in 30-day intervals after volumetric measurements assessed by TC. For each group, 5 urinary bladders were sampled daily from day 1 to 30 and 6 samples every 30 days from day 60 to 360 to detect p50 and p65 activation by IHC.

Immunohistochemical analysis

Excised urinary bladders were fixed in 4% paraformaldehyde (Roti-Histofix 4 % acid-free, pH 7, Roth) by insertion of a transurethral catheter and incubated FOR 48 hours at room temperature. Sagittal halves were embedded in paraffin, and tissue sections of 4 µm were rehydrated and subjected to standard IHC procedure. Antigen retrieval was performed by boiling the slides in citrate buffer, pH 6, at 100°C for 10 minutes. Staining was performed with Vectastain ABC rabbit kit (Vector Laboratories, Burlingame, CA). Primary antibodies, purchased from Abcam (Cambridge, MA) were incubated at 4°C overnight and diluted with Tris-buffered saline to the following concentrations: p50 (ab7971, rabbit monoclonal) to 1:500 and p65 (ab7970, rabbit monoclonal) to 1:500. 3,3-Diaminobenzidine chromogen (Vector Laboratories) was used for signal precipitation, and nuclei were counterstained with hematoxylin.

Antigen localization and intensity were determined with an Olympus light microscope at 400× magnification. Urothelial cells were counted in 5 sample-representing fields using an optical grid (250 µm per field). NF-κB activation was defined by the fraction of marker-positive nuclei (brown) and the corresponding staining intensity.

Transurethral cystotonometry

To determine the intravesical volume, a saline-filled transurethral catheter (0.7 mm diameter IVC cannula, BD NeoflonTM, Helsingborg, Sweden) was inserted into the emptied bladder. The catheter was linked to an infusion pump and to a pressure transducer by pressure-resistant tubes. The saline instillation rate was set to 0.1 mL/min, the transduced signal was detected, and a chart recorder used to determine the intravesical volume at 10 mm Hg.

Micturition frequency analysis

Groups of 5 mice were analyzed simultaneously. Each mouse was set into a single cell with a permeable grid floor allowing the collection of murine excretions on an absorbent paper that moved approximately 15 cm/h for 24 hours. Food and water was provided ad libitum. Micturition spots were detected on an ultraviolet plate, and pictures were processed by ImageJ software to determine the micturition frequency.

Statistical analysis and endpoints

Statistical analysis was performed by using SPSS software version 24 and GraphPad Prism 7.00. For IHC, 5 or 6 animals were analyzed per sampling day to obtain a mean and standard deviation of positively stained nuclei for each animal. Statistical significance between the treatment groups and sampling days was calculated by 2-way analysis of variance and Tukey post hoc testing. Functional analysis based on the volumetric endpoint (>50% reduction of intravesicular bladder volume at 10 mm Hg) was determined by TC. Dose-response curves were calculated by probit analysis of 5 dose groups with 12 mice each. A logarithmic function without a threshold dose was assumed. Treatment effects were evaluated by ED_{50} values (estimated dose at which 50% of animals show radiation response as defined by the volumetric endpoint), and their standard deviation applying likelihood-ratio analysis was based on the logit model. For Kaplan-Meier curves, volumetric endpoint and micturition frequency endpoints (>1 micturition event per hour) were used to generate complication-free survival curves. Log-rank test statistics and the hazard ratio were calculated in GraphPad Prism to determine treatment effects. A P value of $<.05$ was regarded as statistically significant.

Results

Early radiation-induced activation of NF-κB

IHC demonstrated that the high single dose of 23 Gy induced a biphasic activation of NF-κB proteins p50 and p65 (Fig. 1) in ERC (day 1-30). The time courses of p50 and p65 (Fig. 1a, black) show 2 activation peaks but differ in the residual amplitude of activation. p50 is activated in 2 well-defined peaks around day 6 and 21, separated by an inactive phase showing p50 levels similar to not irradiated control values. Compared with p50, p65 shows 2 expanded and flattened peaks that are intermediately decreased around day 12.

NF-κB appears to be activated in 2 phases, from day 1 to 15 (phase 1) and 16 to 30 (phase 2). Systemic administration of thalidomide in both early phases efficiently inhibited the activation of both NF-κB proteins (Fig. 1a). Phase 1 application significantly ($P < .001$) lowered the activation

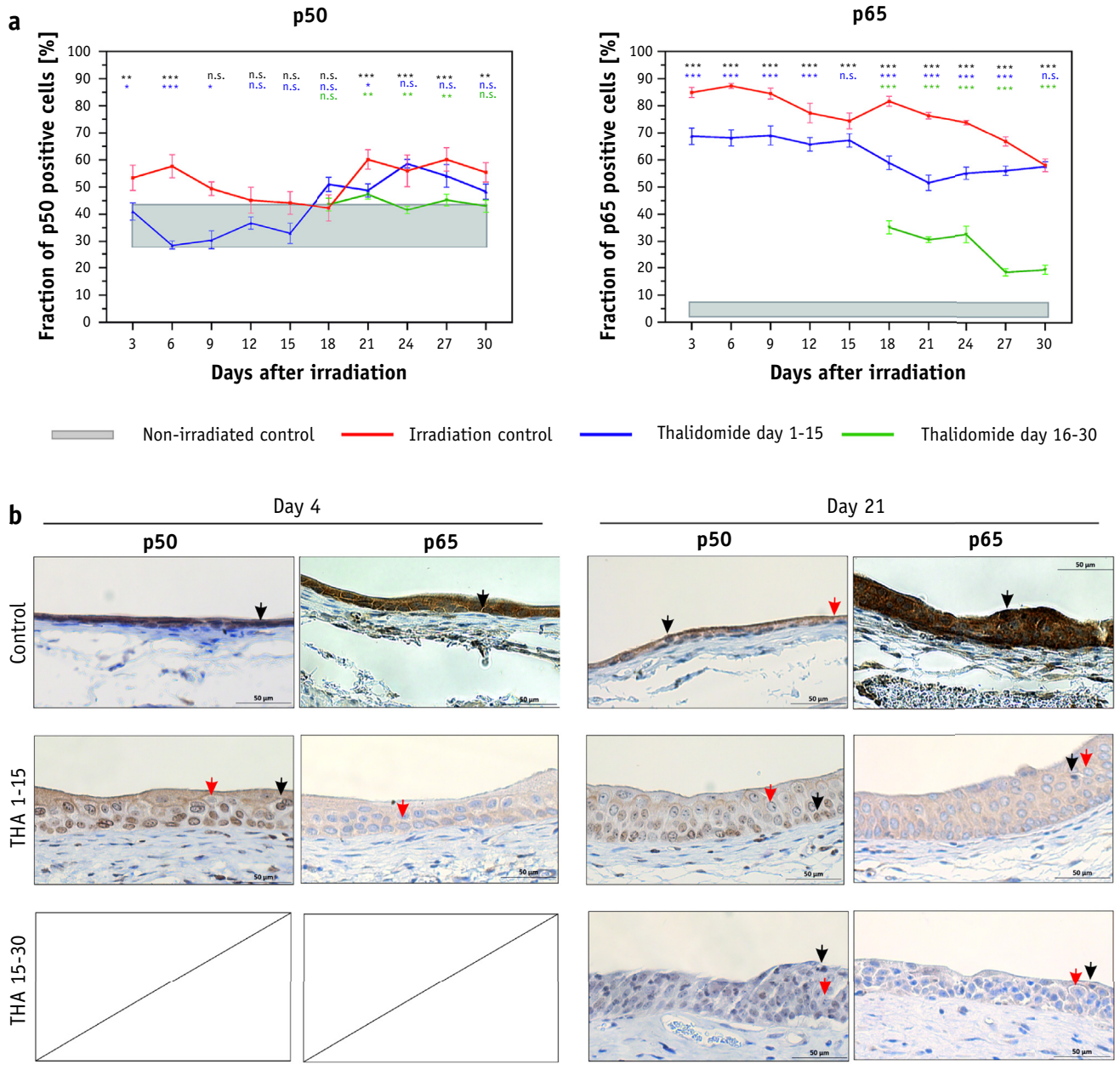

Fig. 1. Involvement of nuclear factor-kappa B (NF-κB) in the pathogenesis of early radiation cystitis (ERC), day 1 to 30. (a) p50 and p65 activation course in response to single irradiation (23 Gy). Each data point (n = maximum of 15 animals) reflects an average of 3 successive time points (daily sampling, n = maximum of 5 animals). The nonirradiated control represents the mean activation and corresponding standard error of mean (SEM) of all samples from day 1 to 30 (n = 50 animals). For 2-way analysis of variance (ANOVA), the irradiation control was compared with the following treatment groups: nonirradiated control (black stars), thalidomide day 1 to 15 (blue stars), and thalidomide day 16 to 30 (green stars). (b) Immunohistochemistry (IHC) illustrates marker localization in the urothelium including the representative staining of p50 and p65 on day 4 and 21 after irradiation alone or in combination with thalidomide application. For analysis, the fraction of positive cells/nuclei (black arrows) was determined. Red arrows indicate no marker staining. $*P < .05$, $**P < .01$, $***P < .001$; scale bar = 50 μm. (A color version of this figure is available at https://doi.org/10.1016/j.ijrobp.2020.01.028.)

of both proteins; however, the time course demonstrated that NF-κB is slightly activated after thalidomide administration. Phase 2 application interfered with activation of NF-κB after day 16 and showed a mitigating effect several days after the onset of treatment. Histologic analysis (Fig. 1b) illustrates that thalidomide in both phases reduced the frequency of positively stained nuclei.

The correlation of functional impairment (Fig. 2a) with the time courses of NF-κB activation suggests that the induction of p50 and p65 precedes the radiogenic reduction

of urinary bladder volume. Both administrations resulted in a drastic decrease in responding animals. In particular, phase 1 treatment from day 1 to 15 reduced the incidence of responding animals to approximately 25% of the irradiated control.

Late radiation-induced activation of NF-κB

In contrast to ERC (day 1-30), the activation time course and intensity of p50 were different from p65 in the chronic response phase (day 60-360). The activation of p65 (Fig. 3a) was maintained at slightly increased levels, whereas the relative fraction of active p50 continuously increased from its minimum on day 60 to reach a plateau starting on day 150. Phase 1 and 2 applications of thalidomide attenuated the activation of p50 and postponed the active plateau phase from day 150 to day 270. p65 is not affected by thalidomide, indicating that it is not involved in the late radiopathogenesis.

Thalidomide was applied from day 150 to 180 (phase 3) to investigate effects on the late activation course and amplitude of p50 and p65. Concomitant with previous results, p65 is not affected and p50 appears to be slightly reduced during the administration period (Fig. 3b). This effect is not persistent; p50 becomes rapidly reactivated afterward and demonstrates a time course similar to that in the irradiated control group. Histologic data show the enduring inhibition effect of early thalidomide administration on day 150 (Fig. 3c).

Functional consequences of thalidomide application on early and late radiogenic sequelae

The therapeutic effect of thalidomide on ERC and LRS was evaluated by dose-effect relationships based on volumetric measurements. Phase 1 and 2 application of thalidomide lowered the levels of active NF-κB (Fig. 1) and decreased the fraction of early responding animals (Fig. 2a). Dose-response analysis (Table 1, Fig. 2b) revealed that phase 1 treatment significantly increased radiation tolerance by doubling the ED_{50} for early responses from 11.6 ± 6.8 Gy to 24.3 ± 2.9 Gy. Phase 2 treatment also affected the ED_{50}, but it was not as pronounced as it was with phase 1 application.

Nevertheless, both early treatments significantly modified the development of the LRS (Fig. 4). The ED_{50} of the

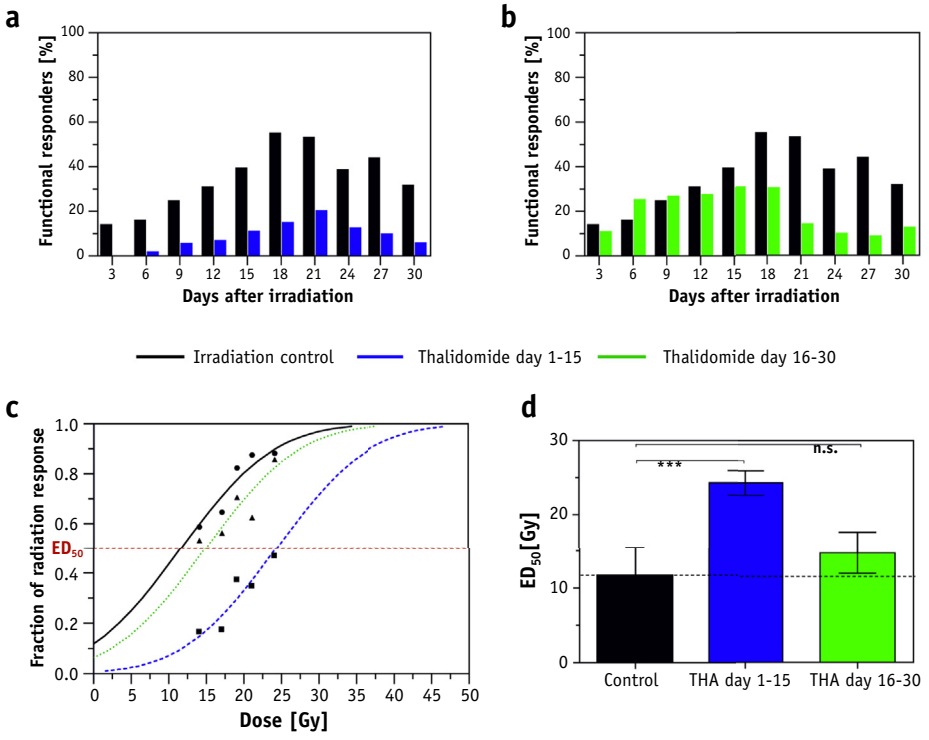

Fig. 2. Functional modifications of daily thalidomide administration on early radiation cystitis (ERC). (a, b) The time course of radiation-induced functional impairment is defined by an individual >50% reduction of urinary bladder compliance (volumetric endpoint) assessed by transurethral cystotonometry (TC) after single irradiation on day 0. The fraction of responders in merged dose groups is illustrated. (c, d) Dose-response curves were generated by probit analysis correlating with radiation response, based on the volumetric endpoint, with the applied dose (14, 17, 19, 21, or 24 Gy). Treatment effects were determined by comparison of corresponding 50% effective dose (ED_{50}) values (± standard deviation [SD]), applying the likelihood ratio analysis based on the logit model. Radiation response (volumetric endpoint) was determined in 3-day intervals between day 3 and 30. $***P < .001$, n = 60 animals.

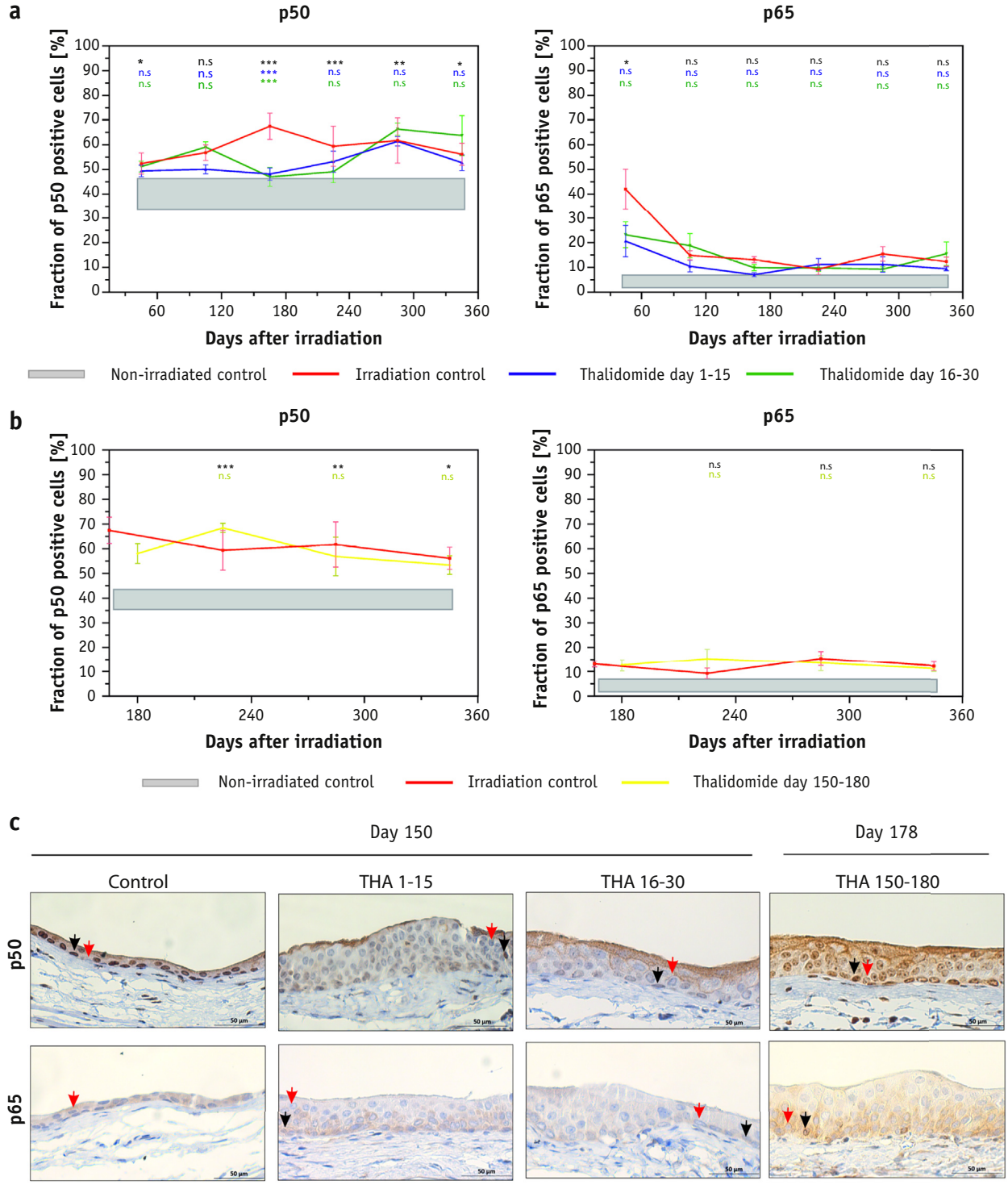

Fig. 3. Role of nuclear factor-kappa B (NF-κB) in chronic radiation sequelae of the urinary bladder, day 60 to 360. (a, b) p50 and p65 activation course in response to single irradiation (23 Gy). One data point (n = maximum of 12 animals) reflects an average of 2 successive time points (30-day intervals, n = maximum of 6 animals). The nonirradiated control represents the mean activation and corresponding standard error of mean (SEM) of all samples from day 30 to 360 (n = 60 animals). For 2-way analysis of variance (ANOVA) analysis, the irradiation control was compared with the following treatment groups: nonirradiated control (black stars), thalidomide day 1 to 15 (blue stars), thalidomide day 16 to 30 (green stars), and thalidomide day 150 to 180 (yellow stars) treatment group. (c) Immunohistochemistry (IHC) illustrates marker localization in the urothelium including the representative staining on day 150. The inhibitory effect of late thalidomide administration is shown on day 178. For analysis, the fraction of positive cells/nuclei (black arrows) was determined. Red arrows indicate no marker staining. $*P < .05$, $**P < .01$, $***P < .001$; scale bar = 50 μm. (A color version of this figure is available at https://doi.org/10.1016/j.ijrobp.2020.01.028.)

Table 1 Summary of ED_{50} values and treatment effects of thalidomide

	Dose-response curve		Comparison to irradiation control
	ED_{50} ± SD, Gy	*P* dose	*P* value
Early phase (day 1-30)			
Irradiation control	11.6 ± 6.8	.009	-
Thalidomide 1-15	24.3 ± 2.9	.0014	.001
Thalidomide 16-30	14.9 ± 4.8	<.001	.388
Late phase (day 60-360)			
Irradiation control	10.4 ± 3.9	<.001	-
Thalidomide 1-15	19.6 ± 2.5	<.001	<.001
Thalidomide 16-30	17.1 ± 2.8	<.001	.01
Thalidomide 150-180	14.2 ± 3.0	<.001	.158

Abbreviations: ED = effective dose; SD = standard deviation. n = 60 animals, 5 dose groups (14-24 Gy). *P* dose refers to the dose-effect relationship, and *P* value determines the treatment effect.

irradiated control group increased from 10.4 ± 3.9 Gy to 19.6 ± 2.5 Gy and 17.1 ± 2.8 Gy in response to phase 1 and 2 thalidomide treatment, respectively. Late administration of thalidomide resulted in minor changes in the ED_{50}. The inefficiency of late treatment was already suggested by mechanistic studies demonstrating that thalidomide has no long-lasting effects on chronic NF-κB (p50) activation.

The ameliorating effect of early thalidomide administration was also demonstrated by the complication-free survival analysis using Kaplan-Meier curves (Fig. 4a, 4b, Table 2). Regarding the volumetric endpoint (Fig. 4a), thalidomide postponed the median incidence of the late response from day 150 to day 300 (phase 1 and 2 treatment). Phase 3 administration showed no relevant modifications. The micturition frequency analysis endpoint (Fig. 4b), revealed to be less sensitive as functional response, was drastically decreased compared with the volumetric endpoint. We assume that the generally reduced functional response potential hid treatment effects.

Discussion

Histologic analyses of irradiated urinary bladders demonstrated a biphasic radiation-induced activation of p50 and p65 in ERC (day 1-30). The formation of a p50/p65 heterodimer emphasizes that early symptoms are based on inflammatory processes.[18,21,23] p50/p65 is activated by the canonical pathway, leading to the upregulation of inflammatory genes. The biphasic time course correlates with previously demonstrated COX-2 activation that is regulated by NF-κB and drives the prostaglandin synthesis, contributing to early adverse effects. The relationship between the time course of NF-κB activation and functional impairment reveals that functional responses manifest with a certain delay after the onset of inflammatory processes, suggesting the involvement of further pathways.

During the chronic phase, only p50 was revealed to be constantly activated, reaching an expression plateau on day 150. p50 homodimers are associated with the expression of nitric oxide synthase 2,[27] interleukin-6,[28] and interleukin-10,[29] which are the driving factors for chronic inflammation.[14,27] Thus, we assume that LRS is based on urothelial inflammation, presumably triggered by an incomplete compensation of radiation-induced urothelial cell depletion and a successive impairment of the barrier function. The interplay of a leaky urothelium and chronic inflammation emphasizes the importance of consequential late side effects because these factors are directly modified by the severity of early reactions. This explains the patient-to-patient variation in latency of the symptom-free phase, which is inversely dose dependent. An intense early phase—influenced by inflammation—increases the risk and degree of incomplete barrier repair, which favors the development of late effects and reduces the latent period.

Our data demonstrated that early thalidomide administration effectively attenuated p50 and p65 activation in both the early and late phase. This effect was confirmed in functional studies in which both early thalidomide treatments significantly increased the ED_{50} value for LRS. Furthermore, our data show that phase 1 treatment resulted in the most beneficial radioprotective effects. Considering thalidomide as a therapeutic agent to manage urinary bladder impairment, our data suggest that applications during the early symptomatic phase have beneficial and promising effects on the incidence and severity.

Current therapy approaches comprise bladder irrigations with a wide spectrum of diverse compounds. Alum and formalin target severe hematuria, whereas the instillation of glycosaminoglycans aims to protect the urothelium from mechanical and chemical stress.[2,3,30] Systemic therapy approaches, such as the administration of TCDO/WF10, take advantage of immunomodulatory properties.[31,32] Most systemic and local treatments that showed therapeutic modifications did not affect the development of chronic radiation fibrosis. Hyperbaric oxygen treatment is proposed to reduce tissue fibrosis; several clinical studies have revealed beneficial results, but long-term follow-up elucidated the recurrence of fibrosis and poor clinical response of patients.[33,34] All in all, there is still a lack of appropriate treatments, suggesting thalidomide should be tested in further (pre-)clinical trials for its ability to reduce ERC and LRS.

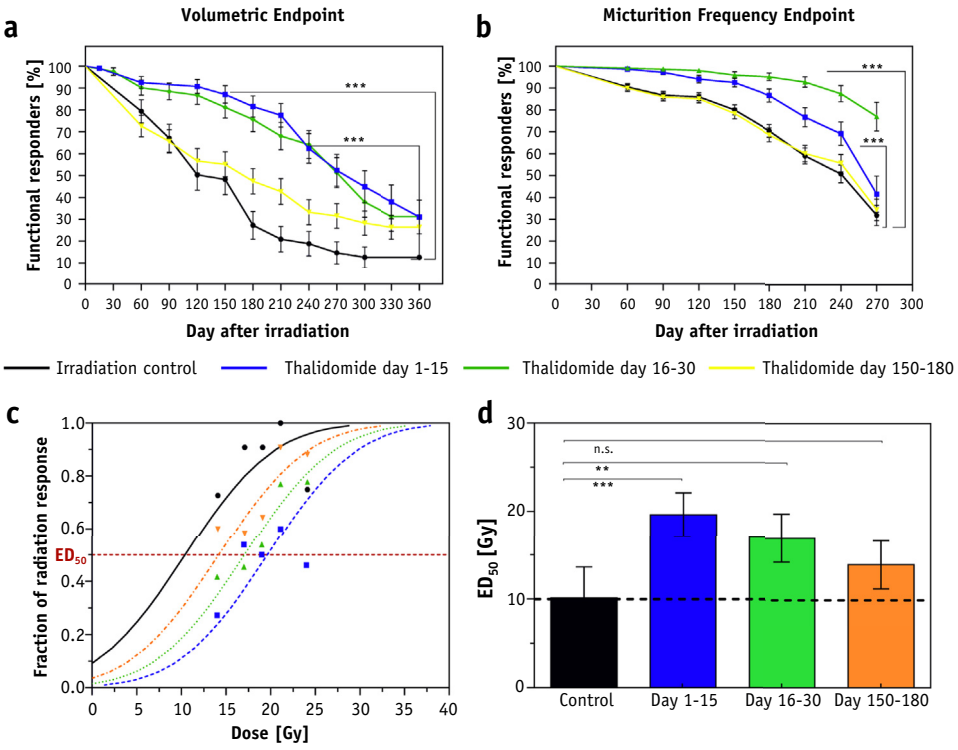

Fig. 4. Functional modifications of thalidomide administration on chronic radiation sequelae. Kaplan-Meier analysis of complication-free survival referred to 2 functional response endpoints: (a) the volumetric endpoint, defined by an individual >50% reduction of bladder compliance (volumetric endpoint), and (b) micturition frequency analysis (MFA), defining a functional response by a micturition frequency of >1 event/hour (micturition frequency endpoint). All dose groups were merged (n = 60 animals). (c, d) Dose-response curves were generated by probit analysis correlating with radiation response, based on the volumetric endpoint, with the applied dose (14, 17, 19, 21, or 24 Gy). Treatment effects were determined by comparison of corresponding 50% effective dose (ED_{50}) values (± standard deviation [SD]), applying the likelihood ratio analysis based on the logit model. Radiation response (volumetric endpoint) was determined in 30-day intervals between day 60 and 360. $***P < .001$, n = 60 animals.

Table 2 Kaplan-Meier complication-free survival

	Mean complication-free survival ± SEM, d	Comparison to irradiation control		
		P value (Mantel-Cox)	Hazard ratio (Mantel-Haenszel)	95% CI of ratio
Volumetric endpoint (>50% reduction of bladder volume)				
Irradiation control	166 ± 13	-	-	-
Thalidomide day 1-15	275 ± 13	<.0001	0.2877	0.173-0.4787
Thalidomide day 16-30	262 ± 13	<.0001	0.343	0.208-0.5664
Thalidomide day 150-180	198 ± 14	.1168	0.6939	0.4395-1.096
Micturition frequency endpoint (>1 event/hr)				
Irradiation control	216 ± 4	-	-	-
Thalidomide day 1-15	243 ± 4	.0001	0.5133	0.3649-0.722
Thalidomide day 16-30	260 ± 3	<.0001	0.2656	0.186-0.3798
Thalidomide day 150-180	216 ± 5	.9065	0.9837	0.7471-1.295

Abbreviations: CI = confidence interval; MFA = micturition frequency analysis; SEM = standard error of the mean.
Analysis based on volumetric endpoint and MFA endpoint. Reciprocal hazard ratio describes treatment efficiency compared with the irradiation control. Merged dose groups; n = 60 animals.

Conclusions

ERC and LRS are based on inflammatory processes in the urothelium. Single irradiation doses activated the NF-κB proteins p50 and p65 during early events, suggesting the formation of heterodimers that induce proinflammatory genes. Contrarily to the early phase, only p50 is activated in chronic reactions, indicating the formation of p50 homodimers that are associated with chronic inflammation.

Thalidomide inhibited p50 and p65 induction significantly and affected the development and severity of early and late adverse effects. Early treatments increased the ED_{50} values for ERS and LRS, whereas the late administration of thalidomide did not show any beneficiary effects.

References

1. Lobo N, Kulkarni M, Hughes S, et al. Urologic complications following pelvic radiotherapy. *Urology* 2018;122:1-9.
2. Smit SG, Heyns CF. Management of radiation cystitis. *Nat Rev Urol* 2010;7:206-214.
3. Zwaans BMM, Chancellor MB, Lamb LE. Modeling and treatment of radiation cystitis. *Urology* 2016;88:14-21.
4. Kolla SB, Dash A. Radiation cystitis: Acute and chronic. In: Radiation Therapy for Pelvic Malignancy and Its Consequences. New York, NY: Springer New York; 2015. p. 111-118.
5. Dörr W, Hendry JH. Consequential late effects in normal tissues. *Radiother Oncol* 2001;61:223-231.
6. Dörr W, Eckhardt M, Ehme A, Koi S. Pathogenesis of acute radiation effects in the urinary bladder. Experimental results. *Strahlenther Onkol* 1998;174:93-95.
7. Jaal J, Dörr W. Radiation induced inflammatory changes in the mouse bladder: The role of cyclooxygenase-2. *J Urol* 2006;175:1529-1533.
8. Di Maggio FM, Minafra L, Forte GI, et al. Portrait of inflammatory response to ionizing radiation treatment. *J Inflamm (Lond)* 2015;12:14.
9. Jaal J, Dörr W. Radiation-induced damage to mouse urothelial barrier. *Radiother Oncol* 2006;80:250-256.
10. Andersson K-E, Arner A. Urinary bladder contraction and relaxation: Physiology and pathophysiology. *Physiol Rev* 2004;84:935-986.
11. Gilmore TD. Introduction to NF-jB: Players, pathways, perspectives. *Oncogene* 2006;25:6680-6684.
12. Magné N, Toillon R-A, Bottero V, et al. NF-κB modulation and ionizing radiation: Mechanisms and future directions for cancer treatment. *Cancer Lett* 2006;231:158-168.
13. Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. *Nat Publ Gr* 2017;2.
14. Yu Y, Wan Y, Huang C. The biological functions of NF-κB1 (p50) and its potential as an anti-cancer target. *Curr Cancer Drug Targets* 2009;9:566.
15. Dolcet D, Llobet J, Pallares X, Matias-Guiu X. NF-kB in development and progression of human cancer. *Virchows Arch* 2005;446:475-482.
16. Li N, Karin M. Is NF-κB the sensor of oxidative stress? *FASEB J* 1999;13:1137-1143.
17. Habraken Y, Piette J. NF-κB activation by double-strand breaks. *Biochem Pharmacol* 2006;72:1132-1141.
18. Wietek C, O'Neill LAJ. Diversity and regulation in the NF-kappaB system. *Trends Biochem Sci* 2007;32:311-319.
19. Hadian K, Krappmann D. Signals from the nucleus: Activation of NF-κB by cytosolic ATM in the DNA damage response. *Sci Signal* 2011;4. pe2-pe2.
20. Zhang Q, Lenardo MJ, Baltimore D. Leading edge review 30 years of NF-kB: A blossoming of relevance to human pathobiology. *Cell* 2017;168:37-57.
21. Smale ST. Dimer-specific regulatory mechanisms within the NF-κB family of transcription factors. *Immunol Rev* 2012;246:193-204.
22. Frings K, Gruber S, Kuess P, Kleiter M, Dörr W. Modulation of radiation-induced oral mucositis by thalidomide. *Strahlentherapie und Onkol* 2016;192:561-568.
23. Gannon PO, Lessard L, Stevens L-M, et al. Large-scale independent validation of the nuclear factor-kappa B p65 prognostic biomarker in prostate cancer. *Eur J Cancer* 2013;49:2441-2448.
24. Eisen T, Boshoff C, Mak I, et al. Continuous low dose thalidomide: A phase II study in advanced melanoma, renal cell, ovarian and breast cancer. *Br J Cancer* 2000;82:812-817.
25. Sherbet GV. Therapeutic potential of thalidomide and its analogues in the treatment of cancer. *Anticancer Res* 2015;35:5767-5772.
26. Dörr W, Beck-Bornholdt H-P, Dorr W. Radiation-induced impairment of urinary bladder function in mice: Fine structure of the acute response and consequences on late effects. *Radiat Res* 1999;151:461.
27. Simon PS, Sharman SK, Lu C, et al. The NF-κB p65 and p50 homodimer cooperate with IRF8 to activate iNOS transcription. *BMC Cancer* 2015;15:770.
28. Kishimoto T. Interleukin 6: Discovery of a pleiotropic cytokine. *Arthritis Res Ther* 2006;8:S2.
29. Cao S, Zhang X, Edwards JP, Mosser DM. NF-κB1 (p50) homodimers differentially regulate proand antiinflammatory cytokines in macrophages, *J Biol Chem* 2006;281:26041-26050.
30. Thompson A, Adamson A, Bahl A, et al. Guidelines for the diagnosis, prevention and management of chemical- and radiation-induced cystitis. *J Clin Urol* 2014;7:25-35.
31. Veerasarn V, Boonnuch W, Kakanaporn C. A phase II study to evaluate WF10 in patients with late hemorrhagic radiation cystitis and proctitis. *Gynecol Oncol* 2006;100:179-184.
32. Sarsarshahi S, Madjd Z, Bozsaky E, et al. An evaluation of the effect of bortezomib on radiation-induced urinary bladder dysfunction. *Strahlentherapie und Onkol* 2019;195:934-939.
33. Chong KT, Hampson NB, Corman JM. Early hyperbaric oxygen therapy improves outcome for radiation-induced hemorrhagic cystitis. *Urology* 2005;65:649-653.
34. Shao Y, Lu G, Shen Z. Comparison of intravesical hyaluronic acid instillation and hyperbaric oxygen in the treatment of radiation-induced hemorrhagic cystitis. *BJU Int* 2012;109:691-694.